Assembly of the Mycobacterial Cell Wall

Monika Jankute, Jonathan A.G. Cox, James Harrison, and Gurdyal S. Besra

School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom; email: g.besra@bham.ac.uk

Annu. Rev. Microbiol. 2015. 69:405–23

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi: 10.1146/annurev-micro-091014-104121

Keywords

arabinogalactan, biosynthesis, cell wall, lipoarabinomannan, mycolic acids, peptidoglycan

Abstract

Mycobacterium tuberculosis remains one of the most successful bacterial pathogens, claiming over 1.3 million lives worldwide in 2013. The emergence of multidrug-resistant and extensively drug-resistant isolates has prompted the need for new drugs and drug targets. *M. tuberculosis* possesses an unusual cell wall dominated by lipids and carbohydrates that provides a permeability barrier against hydrophilic drugs and is crucial for its survival and virulence. This large macromolecular structure, termed the mycolyl-arabinogalactan-peptidoglycan complex, and the phosphatidyl-*myo*-inositol-based lipoglycans are key features of the mycobacterial cell wall. Assembly of these cell wall components is an attractive target for the development of chemotherapeutics against tuberculosis. Herein, we focus on recent biochemical and molecular insights into these complex molecules of *M. tuberculosis* cell wall.

Contents

INTRODUCTION

A hallmark of mycobacteria is their intricate cell wall. Inhibition of cell wall assembly has proven useful for tuberculosis chemotherapy; drugs such as ethambutol, isoniazid, ethionamide, and D-cycloserine successfully target the synthesis of its various components. As a result, a complete understanding of cell wall biosynthesis has been a major research objective over the last decade. The cell envelope of *Mycobacterium tuberculosis* consists of three main structural components: (*a*) the characteristic long-chain mycolic acids, (*b*) a highly branched arabinogalactan (AG) polysaccharide, and (*c*) a cross-linked network of peptidoglycan (**Figure 1**). The entire complex, referred to as mAGP, is essential for cell viability. In addition, an outer membrane segment that contains solvent-extractable lipids, such as inert waxes and glycolipids, intercalates the mycolate layer of the mAGP complex (87–89). Finally, an outermost capsule composed of polysaccharides and proteins completes the cell envelope of *M. tuberculosis*. This review focuses primarily on mycobacterial cell wall core assembly.

PEPTIDOGLYCAN

Structure of Peptidoglycan

Peptidoglycan (PG) is present in almost all bacteria, providing shape, rigidity, and osmotic stability to both gram-negative and gram-positive bacilli (108). All PGs produced by bacteria share the same basic core structure: a glycan backbone and short cross-linked peptide side chains (124). The backbone of PG is typically made up of *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) linked via β(1→4) linkages (21). However, mycobacterial PGs possess one notable structural modification: MurNAc is oxidized to *N*-glycolylmuramic acid (MurNGlyc) (**Figure 2**) (76, 104). This modification is believed to be involved in increasing the overall strength of PG by providing sites for hydrogen bonding, as well as in potentially decreasing susceptibility to lysozyme

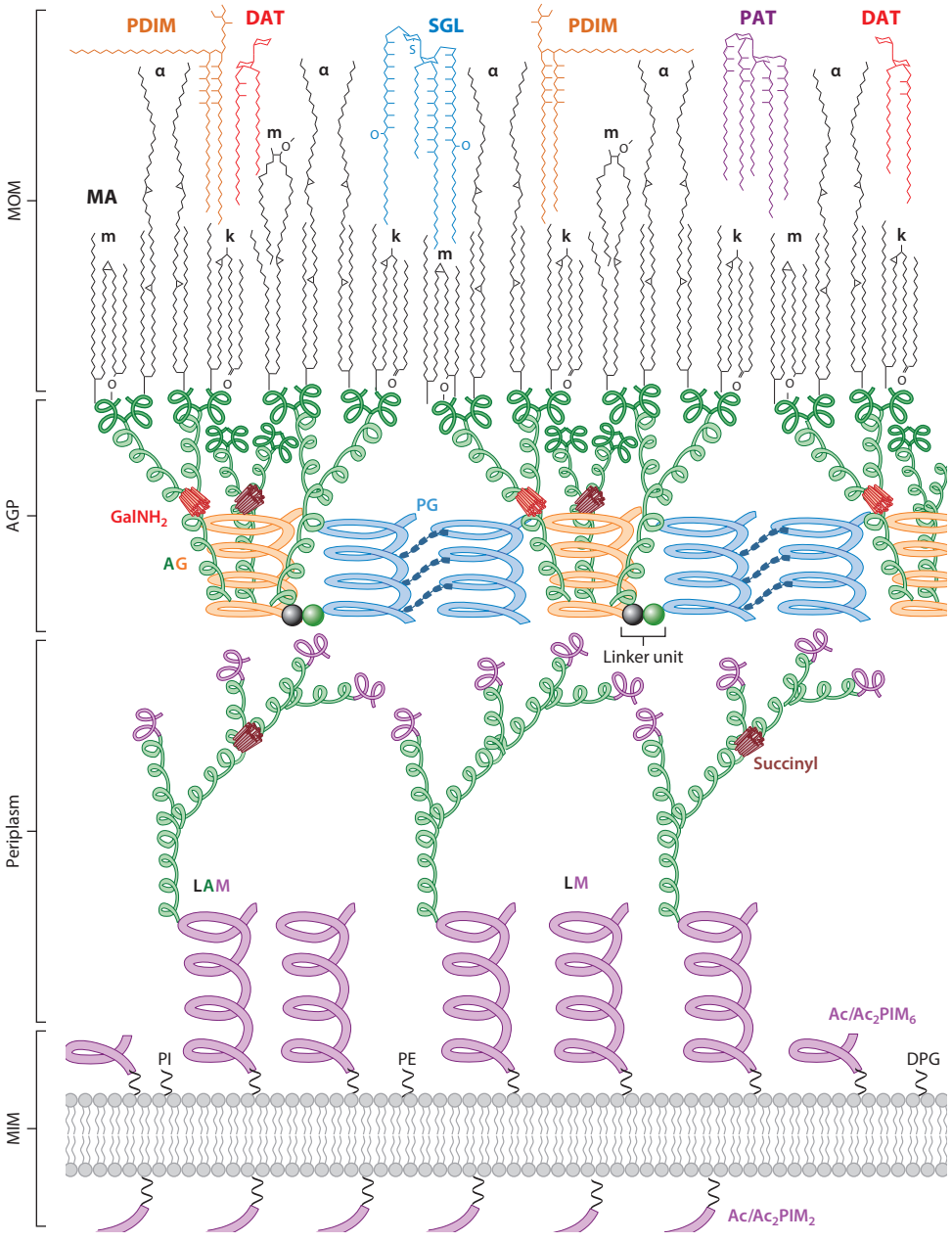

Figure 1

Schematic representation of *Mycobacterium tuberculosis* cell envelope. The capsular material is not represented here. Abbreviations: Ac/Ac_2PIM_2, tri-/tetra-acylated phosphatidyl-*myo*-inositol-dimannoside; Ac/Ac_2PIM_6, tri-/tetra-acylated phosphatidyl-*myo*-inositol-hexamannoside; AG, arabinogalactan; AGP, arabinogalactan-peptidoglycan complex; DAT, diacyltrehalose; DPG, diphosphatidylglycerol; $GalNH_2$, galactosamine residue; k, keto; LAM, lipoarabinomannan; LM, lipomannan; m, methoxy; MA, mycolic acids; MIM, mycobacterial inner membrane; MOM, mycobacterial outer membrane; PAT, polyacyltrehalose; PDIM, phthiocerol dimycocerosate; PE, phosphatidylethanolamine; PG, peptidoglycan; PI, phosphatidyl-*myo*-inositol; SGL, sulfoglycolipid.

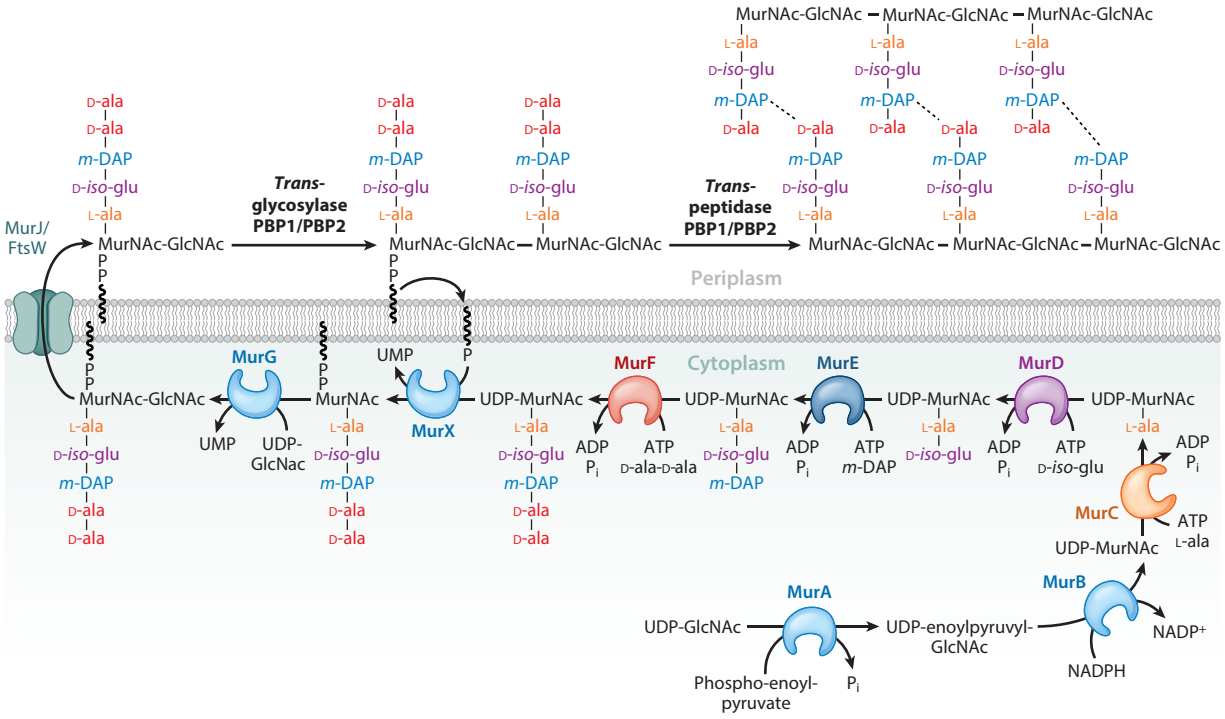

Figure 2

The biosynthesis pathway of peptidoglycan in *Mycobacterium tuberculosis*.

(21, 104). Tetrapeptide side chains are attached to the muramyl components of the backbone that become cross-linked to provide a mesh-like structure. In *M. tuberculosis*, these side chains consist of L-alanyl-D-isoglutaminyl-*meso*-diaminopimelyl-D-alanine that is cross-linked between *meso*-diaminopimelic acid (*m*-DAP) and D-alanine (3→4), which means *M. tuberculosis* has an A1γ-group PG, based on the classification system of Schleifer & Kandler (108). However, mycobacterial PG also has a high number of *m*-DAP to *m*-DAP linkages (3→3), which increase in abundance when the bacilli enter the stationary phase (up to 80% of cross-linkages) (**Figure 2**) (67). *M. tuberculosis* has the ability to modify these cross-linkages from (3→4) to (3→3) without the requirement of de novo synthesis of PG, which could provide the bacilli with protection from endopeptidases (67). Another unique feature of mycobacterial PG is that it provides the site for AG attachment. The 6 position of some muramyl units of the PG backbone provides a site for a phosphodiester bond to an α-L-rhamnopyranose-(1→3)-α-D-GlcNAc-(1→P) disaccharide bridge (78).

Biosynthesis of Peptidoglycan

The sequential Mur ligase pathway is the major contributor to the biosynthesis of PG. MurA (Rv1315) has enoylpyruvyl transferase activity that adds phospho-enol-pyruvate to UDP-GlcNAc to form UDP-enoylpyruvyl-GlcNAc (59). MurB (Rv0482) then utilizes NADPH to reduce the enoylpyruvyl moiety from the product of MurA, to a lactoyl ether moiety, to form UDP-MurNAc (12). NamH (Rv3808) then hydroxylates UDP-MurNAc to UDP-MurNGlyc to provide both types of UDP-muramyl substrates (104). From this point, amino acid residues are

attached to UDP-MurNAc/Glyc by the ATP-dependent Mur ligases, beginning with UDP-*N*-acetylmuramoyl:L-alanine ligation by MurC (Rv2151c). This is followed by D-isoglutamate ligation by MurD (Rv2155c), *m*-DAP addition by MurE (Rv2158c), and finally D-alanyl-D-alanine ligation by MurF (Rv2157c) (**Figure 2**) (97). This produces Park's nucleotide, the muramyl-pentapeptide: UDP-MurNAc/Glyc-L-ala-D-isoglu-*m*-DAP-D-ala-D-ala (65). There have been numerous reviews of the ATP-dependent Mur ligase pathway in recent years (8, 36, 117); for *M. tuberculosis*, however, only MurC (75) and MurE (9) have been biochemically characterized.

Park's nucleotide is transferred to decaprenyl phosphate by MurX (Rv2156c), also known as MraY (19). This gives rise to what is known as Lipid I. MurG (Rv2153c) then attaches GlcNAc from UDP-GlcNAc to Lipid I via a β(1→4) linkage between GlcNAc and MurNAc/Glyc to form Lipid II, the final monomeric unit of PG synthesis (121). There is debate as to the identity of the Lipid II flippase, which translocates Lipid II across the plasma membrane. It was initially reported to be MurJ in *Escherichia coli* (Rv3910) by Ruiz (106), but this was later disputed by Mohammadi et al. (94), who identified FtsW (Rv2154c) as having in vitro flippase activity. However, Ruiz's group (113) has recently reported in vivo data from *E. coli* that lend more credence to the identification of the flippase as MurJ. Nonetheless, more research on both enzymes is needed to fully characterize the mycobacterial Lipid II flippase.

After translocation of Lipid II across the plasma membrane, the bifunctional PonA1/PBP1 (Rv0050) and PonA2/PBP2 (Rv3682) transglycosylate Lipid II monomers by attaching the GlcNAc moiety to the muramyl moiety of the growing PG chain (47). In addition to their transglycosylation domain, PonA1 and PonA2 have transpeptidase activity, which forms the classical (3→4) cross-linkages between *m*-DAP and D-ala, at the expense of cleavage between the D-ala-D-ala peptide side chain (**Figure 2**) (23). Other domains of PonA1 and PonA2 include a transmembrane helix and a PASTA (abbreviation for PBP and serine/threonine-kinase associated) domain. However, it does not behave as a typical PASTA domain. It does not bind the expected ligands; therefore, further characterization of this domain is required (22). When *M. tuberculosis* enters dormancy, the number of (3→3) cross-linkages between *m*-DAP residues increases through PG rearrangement by nonclassical L,D-transpeptidases (28). Five paralogs within *M. tuberculosis* have been identified: LdtMt1 (Rv0116c), LdtMt2 (Rv2518c), LdtMt3 (Rv1433), LdtMt4 (Rv0192), and LdtMt5 (Rv0483). However, only LdtMt1- and LdtMt2-deficient strains have been shown to display phenotypic differences in PG structure (110). The L,D-transpeptidases perform the conversion from (3→4) to (3→3) cross-linking by cleaving the remaining D-ala residue from the donor chain, leaving behind a tripeptide chain in the stationary phase PG (107).

ARABINOGALACTAN

Structure of Arabinogalactan

This highly branched macromolecule is composed predominantly of galactose (Gal) and arabinose (Ara) sugar residues, both in the furanose (*f*) ring form (80). In *M. tuberculosis*, about 10–12% of the MurNGlyc residues of PG are covalently attached to AG via a specialized linker unit, α-L-Rha*p*-(1→3)-α-D-GlcNAc-(1→P) (78). Specifically, this linker unit connects the galactan domain of AG to the C-6 position of selected MurNGlyc residues (78). The galactan component of AG is composed of a linear chain of approximately 30 alternating 5- and 6-linked β-D-Gal*f* residues (30). The highly branched arabinan chains, each containing roughly 30 Ara*f* residues, are connected to the linear galactan at C-5 of some of the β(1→6) Gal*f* residues (14). Previous studies in *M. tuberculosis* and in the closely related *Corynebacterium glutamicum* suggested that three branched-chain arabinan units are attached to the galactan chain at positions 8, 10, and 12 (4, 16,

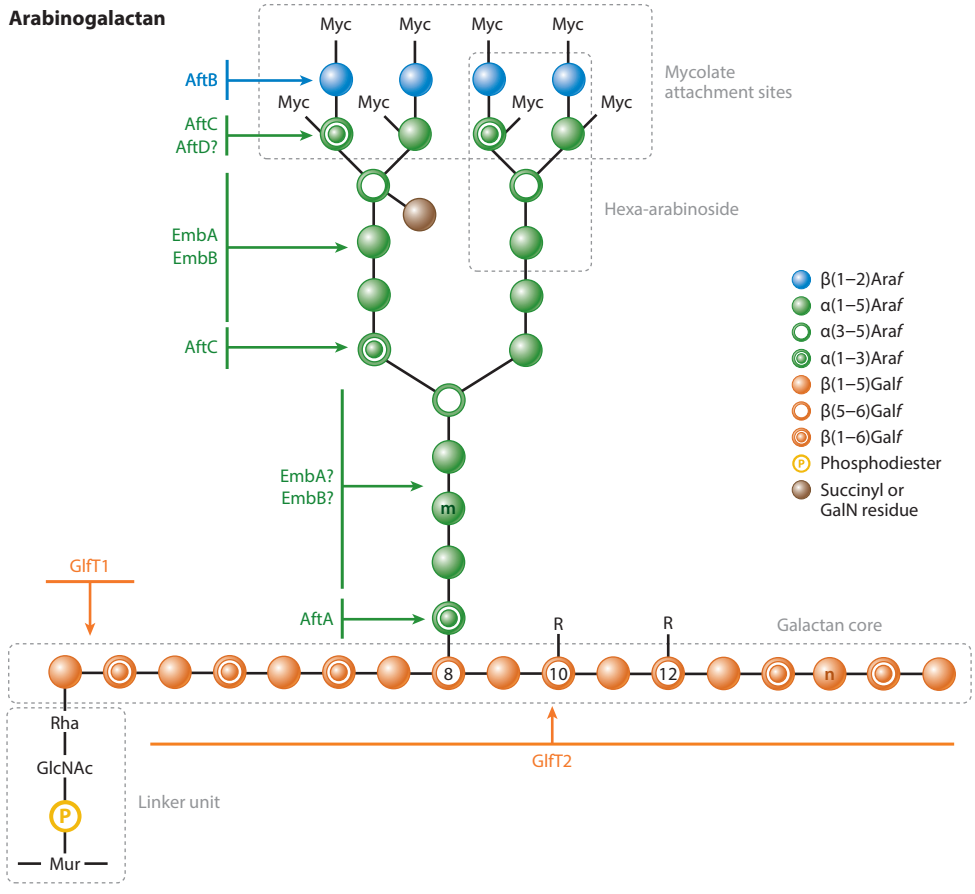

Figure 3

Structure of mycobacterial arabinogalactan and roles of key enzymes that are responsible for its biosynthesis. Adapted from Reference 51 with permission from Future Medicine.

69). However, recently it has been proposed that only two arabinan units are attached per galactan chain (15). The inner core of the arabinan domain contains a backbone of α-5-linked α-D-Ara*f* residues with key branching introduced by 3,5-α-D-Ara*f* residues (30). Galactosamine (D-GalN) and succinyl substituents were identified on the C-2 position of some inner 3,5-α-D-Ara*f* residues (16, 34, 69, 102). It was estimated that one D-GalN residue per AG is present exclusively in the walls of slow-growing mycobacteria and that up to three succinyl esters per AG are present in both slow- and fast-growing mycobacterial species. Interestingly, only nonmycolated arabinan chains were succinylated, and none of the decorated arabinan chains possessed both substituents (16). The nonreducing termini of the arabinan unit consist of a characteristic hexa-arabinoside motif, [β-D-Ara*f*-(1→2)-α-D-Ara*f*]$_2$-3,5-α-D-Ara*f*-(1→5)-α-D-Ara*f*, where position 5 of both the terminal β-D-Ara*f* and the penultimate 2-α-D-Ara*f* serves as an attachment site for mycolic acids (79). In *M. tuberculosis*, mycolyl units were shown to cluster in groups of four and occupy only about two-thirds of the available attachment sites on the terminal hexa-arabinoside motif (79). These structural features are summarized in **Figure 3**.

Biosynthesis of Arabinogalactan

The biosynthesis of AG begins on the cytoplasmic side of the plasma membrane with the formation of the linker unit that anchors the AG component to PG. WecA (Rv1302) initiates the transfer of GlcNAc-1-P to decaprenyl-phosphate (C_{50}-P) to form C_{50}-P-P-GlcNAc (53, 85). The linker unit is completed by the rhamnosyltransferase WbbL (Rv3265c), which transfers Rha from dTDP-Rha to the 3 position of GlcNAc of C_{50}-P-P-GlcNAc, yielding C_{50}-P-P-GlcNAc-Rha (85, 86). The linker unit then serves as an acceptor for the cytoplasmic polymerization of the linear galactan chain. GlfT1 (Rv3782) and GlfT2 (Rv3808c), both of which utilize UDP-Gal*f*, have been identified as bifunctional galactofuranosyltransferases (Gal*f*T) responsible for the synthesis of the galactan chain. Specifically, GlfT1 transfers a Gal*f* residue to the C-4 position of Rha and a second Gal*f* residue to the C-5 position of the first Gal*f*, resulting in C_{50}-P-P-GlcNAc-Rha-Galf_2 (1, 10, 83). The second enzyme, GlfT2, completes the synthesis of the galactan chain by the sequential addition of Gal*f* residues in alternating β(1→5) and β(1→6) linkages, resulting in the production of C_{50}-P-P-GlcNAc-Rha-Galf_{30}. (63, 105, 129). Recent studies have proposed a processive polymerization mechanism by which GlfT2 controls the length of the galactan chain (71, 77). It is believed that subsequent steps in AG assembly, including arabinan polymerization, take place on the outside of the plasma membrane. Despite investigations into the nature of transport of mycobacterial cell wall polysaccharides, proteins involved in these processes remain largely unknown (20, 32). Chemical analysis of the mature lipid-linked galactan synthesized in vitro suggests that Ara*f* residues are transferred directly onto C_{50}-P-P-GlcNAc-Rha-Galf_{30}, utilizing decaprenyl-monophosphoryl-D-arabinose (DPA) (130). DPA formation is catalyzed by phosphoribosyl-1-pyrophosphate synthetase PrsA (Rv1017c), decaprenylphosphoryl-5-phosphoribose synthase UbiA (Rv3806c), and a putative phospholipid phosphatase Rv3807c to form DPR. Decaprenylphosphoribose 2′ epimerase composed of DprE1 (Rv3790) and DprE2 (Rv3791) subunits then catalyzes the epimerization of the ribosyl unit of DPR, resulting in DPA (3, 4, 52, 82, 84). Recent studies described a transporter Rv3789, which appears to reorientate DPA to the periplasm, thus positioning this arabinose donor to be utilized in the synthesis of the arabinan domain of both AG and lipoarabinomannan (LAM) (66). The number of Ara*f*Ts involved in the assembly of the arabinan domain in AG remains a matter of speculation, but the current structure of AG suggests at least six different Ara*f*Ts. Deletion studies in *C. glutamicum*, where AG is dispensable, determined that AftA (Rv3792) transfers three single Ara*f* residues to the 8th, 10th, and 12th Gal*f* residues of C_{50}-P-P-GlcNAc-Rha-Galf_{30} (5). It is speculated that further α(1→5) polymerization of the arabinan domain is catalyzed by EmbA (Rv3794) and EmbB (Rv3795). Deletion of the singular *emb* gene from *C. glutamicum* demonstrated severe reduction of arabinose, resulting in a truncated AG structure with only terminal Ara*f* residues, consistent with this hypothesis (4). Both EmbA and EmbB enzymes were also shown to play a role in forming the characteristic hexa-arabinofuranoside motif (37). Deletion studies in *Mycobacterium smegmatis* identified a branching enzyme AftC (Rv2673) that was responsible for the α(1→3) branching of the internal arabinan domain of both AG and LAM (17, 18). AftD was proposed as a second α(1→3)-branching enzyme involved in the synthesis of AG and LAM; however, its precise function in the assembly of these components remains to be determined (116). Finally, AftB was shown to catalyze the transfer of the terminal β(1→2) Ara*f* residues from DPA to the terminal hexa-arabinofuranoside motif at the nonreducing end of AG (111).

The addition of succinyl and GalN substituents to the inner regions of arabinan completes the primary structure of AG (16, 34, 115). Although enzymes involved in the transfer of the succinyl residues remain unknown, key components required for the synthesis and addition of the GalN substituent have been recently elucidated (115). PpgS (Rv3631) catalyzes the formation of polyprenol-P-D-GlcNAc, which is deacetylated by an unknown deacetylase, before or after

translocation across the plasma membrane. Rv3779 then transfers the GalN unit to the C-2 position of the 3,5-branched Ara*f* residue of AG (115). It is speculated that the protonated D-GalN may interact with anionic substances, such as negatively charged succinyl residues or phosphates of glycolipids, thus contributing to the rigidity and tightness of the AG structure (16, 34). Moreover, the D-GalN unit, which is found only in slow-growing mycobacteria, was proposed to serve a role during host infection (115). However, the precise function of both D-GalN and succinyl residues remains to be established. Very little evidence has been obtained on how or when mycolic acids are directly transferred onto the AG. In vitro enzyme assays in combination with site-directed mutagenesis studies identified the antigen 85 complex as responsible for the transfer of mycolic acids onto trehalose, yielding trehalose monomycolates (TMM) and trehalose dimycolates (TDM) (11, 48). Inactivation of antigen 85 in *M. tuberculosis* led to reduced transfer of mycolates to the mycobacterial cell wall (48). However, the precise mechanistic details of their involvement in AG mycolation remains to be firmly established.

PHOSPHATIDYL-*MYO*-INOSITOL MANNOSIDES, LIPOMANNAN, AND LIPOARABINOMANNAN

Structure of Phosphatidyl-*myo*-Inositol Mannosides, Lipomannan, and Lipoarabinomannan

These noncovalently linked glycophospholipids [phosphatidyl-*myo*-inositol mannosides (PIMs) and their more related glycosylated end products, lipomannan (LM) and lipoarabinomannan (LAM)] are abundant in the inner and outer membranes of all *Mycobacterium* species (39, 101, 103). In conjunction with their physiological function, these distinct glycoconjugates play a key role in modulating the host immune response during infection (74, 92, 100, 109). PIMs are unique glycolipids composed of a phosphatidyl-*myo*-inositol (PI) unit, one to six α-D-mannopyranosyl (Man*p*) residues, and up to four acyl chains (**Figure 4**). The PI unit is based on a *sn*-glycero-3-phospho-(1-D-*myo*-inositol) that is further glycosylated with single Man*p* residues at the O-2 and O-6 positions of *myo*-inositol resulting in a mannosyl phosphate inositol (MPI) anchor (6, 7, 39, 98, 112). The structure of the MPI anchor is highly heterogeneous, with variations in the number, location, and nature of acyl chains. Four potential sites of acylation are available on the MPI anchor: 1-OH and 2-OH of the glycerol unit, 3-OH of the *myo*-inositol residue, and 6-OH of the Man*p* residue attached to *myo*-inositol at the O-2 position (58). The most abundant species of PIMs in mycobacteria are the tri- and tetra-acylated phospho-*myo*-inositol dimannosides (Ac_1PIM_2 and Ac_2PIM_2, respectively) and tri- and tetra-acylated phospho-*myo*-inositol hexamannosides (Ac_1PIM_6 and Ac_2PIM_6, respectively). Both Ac_1PIM_2 and Ac_1PIM_6 contain two acyl groups on the glycerol moiety and an additional acyl group at either the 3-OH of *myo*-inositol or the 6-OH of the Man*p* attached to the O-2 position of *myo*-inositol, whereas Ac_2PIM_2 and Ac_2PIM_6 possess four acyl groups at all of these positions. In mycobacteria, palmitic and tuberculostearic acyl chains are the most abundant substituents (6, 40, 70).

In all *Mycobacterium* species, LM and LAM both possess a mannan chain that contains approximately 21–34 α(1→6)-linked Man*p* residues periodically decorated with 5–10 units of single α(1→2)-Man*p* residues (**Figure 4**) (25, 56). Notably, in the case of *Mycobacterium chelonae*, branching occurs at the C-3 position (44). The length of the mannan chain and the degree of branching in LM are species specific. The mannan core is further glycosylated with 55–72 Ara*f* residues, resulting in LAM. The arabinan domain contains a single linear α(1→5)-linked Ara*f* chain with 3,5-α-D-Ara*f* branches (17, 56). The nonreducing end of the arabinan domain of LAM terminates in either a branched hexa-arabinoside {[β-D-Ara*f*-(1→2)-α-D-Ara*f*]$_2$-3,5-α-D-Ara*f*-(1→5)-α-D-Ara*f*]} or a

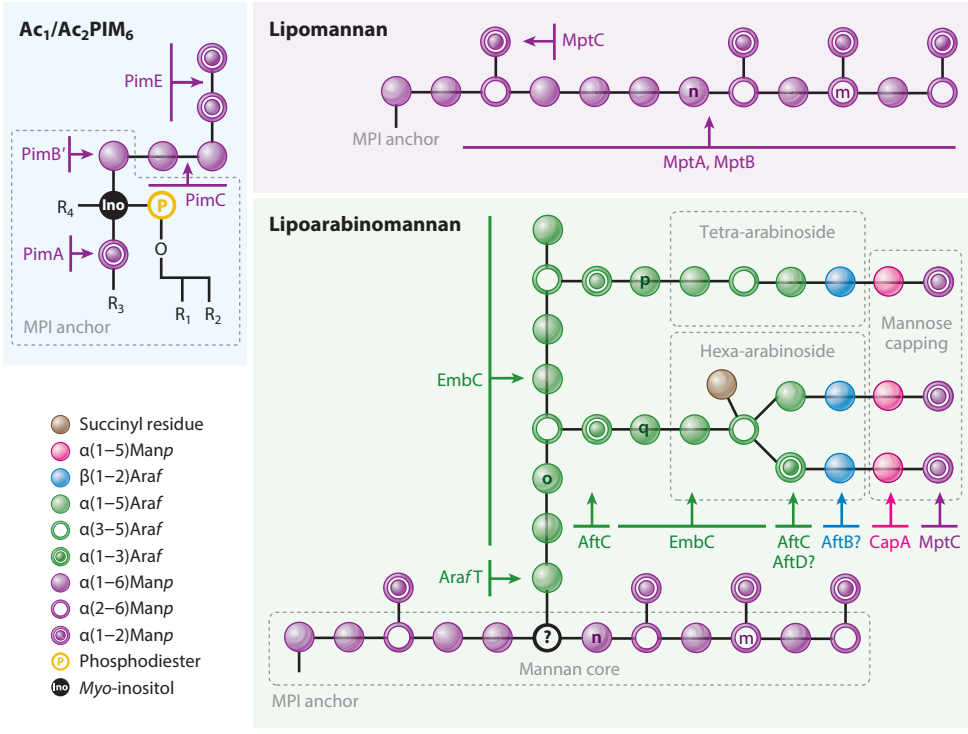

Figure 4

Structure of mycobacterial tri-/tetra-acylated phosphatidyl-*myo*-inositol hexamannoside (Ac_1/Ac_2PIM_6), lipomannan and lipoarabinomannan, and roles of key enzymes that are responsible for their biosynthesis. Abbreviation: MPI, mannosyl phosphate inositol. Adapted from Reference 51 with permission from Future Medicine.

linear tetra-arabinoside [β-D-Ara*f*-(1→2)-α-D-Ara*f*-(1→5)-α-D-Ara*f*-(1→5)-α-D-Ara*f*] (24, 26, 81). The arabinan motifs of LAM are further decorated by capping moieties. *M. tuberculosis* and *Mycobacterium leprae* modify the arabinan termini with a series of α(1→2)-linked Man*p* residues (ManLAM), whereas *M. smegmatis* possesses phosphoinositol caps (PILAM) (27, 57, 99). The absence of capping moieties (AraLAM) in LAM has been shown in *M. chelonae* (44). In addition, a unique 5-deoxy-5-methylthio-xylofuranose (MTX) substituent linked at C-4 of the terminal Man*p* of the ManLAM cap was identified in *M. tuberculosis* (54, 119, 122, 123). Interestingly, a similar MTX residue was also identified in the ManLAM of *Mycobacterium kansasii*; however, the substituent was located on the mannan core instead of the capping Man*p* residues (43). Finally, similarly to AG, succinyl groups were identified on the C-2 position of some inner 3,5-α-D-Ara*f* residues (31).

Biosynthesis of Phosphatidyl-*myo*-Inositol Mannosides, Lipomannan, and Lipoarabinomannan

The early steps of PIM biosynthesis occur in the cytoplasm and employ GDP-Man*p* as the mannose donor. PimA (Rv2610c), an α-mannopyranosyltransferase (Man*p*T), initiates the transfer of a single Man*p* residue from GDP-Man*p* to the 2 position of the *myo*-inositol ring of PI to yield PIM_1 (46, 60). PIM_1 is then further glycosylated by PimB′ (Rv2188c), which transfers the second

single Man*p* to the 6 position of the *myo*-inositol ring, generating PIM_2 (45, 68, 91). This second mannosylation step may occur before or after acylation of PIM_1 at the C-6 position of the Man*p* residue linked to the 2-OH position of *myo*-inositol, catalyzed by the acyltransferase Rv2611c (61). In vitro enzyme assays utilizing PimA and PimB′ of *M. smegmatis* demonstrated preference in generating $AcPIM_2$ over $AcPIM_1$, indicating preference of mannosylation over the acylation step (45). However, deletion of *pimB′* in *C. glutamicum* resulted in accumulation of $AcPIM_1$, suggesting that acylation of PIM_1 is favored over the second mannosylation step (68, 91). The acyltransferase responsible for the transfer of the fourth acyl chain to the C-3 position of the *myo*-inositol ring, thus forming Ac_2PIM_1/Ac_2PIM_2, remains to be established. Previous studies have shown that RvD2-*ORF1* from *M. tuberculosis* CDC1551, designated as PimC, catalyzes further mannosylation of Ac_1/Ac_2PIM_2, resulting in Ac_1/Ac_2PIM_3 (64). However, no strong homologs of *pimC* have been identified in *M. tuberculosis* H37Rv or *M. smegmatis*. PimC or a yet-unidentified $\alpha(1\rightarrow6)$ Man*p*T [PimD] catalyzes the subsequent addition of a Man*p* residue to the nonreducing end of Ac_1/Ac_2PIM_3, resulting in Ac_1/Ac_2PIM_4. Formation of Ac_1/Ac_2PIM_4 marks a branch point in the biosynthesis toward the synthesis of higher PIMs, LM, and LAM (95, 96). Subsequent steps are predicted to occur in the periplasmic space and to employ GT-C glycosyltransferases that utilize polyprenyl-phosphate-based mannose donors (PPM) in contrast to the GT-A/B superfamily, which employs nucleotide-derived sugars (13, 73). The transporters that are responsible for flipping PIMs across the plasma membrane remain to be identified. PimE (Rv1159) catalyzes the $\alpha(1\rightarrow2)$ addition of a Man*p* residue to the Ac_1/Ac_2PIM_4, resulting in formation of Ac_1/Ac_2PIM_5 (96). It is not clear whether the transfer of a second Man*p* residue, thus generating Ac_1/Ac_2PIM_6, is catalyzed by PimE or by a yet-unidentified, putative $\alpha(1\rightarrow2)$ Man*p*T of the GT-C superfamily, which utilizes PPM (96). A parallel pathway employs Ac_1/Ac_2PIM_4 intermediates for the assembly of LM and LAM. Recent studies demonstrated that LpqW (Rv1166) regulates this branch point and promotes the channeling of Ac_1/Ac_2PIM_4 toward LM and LAM biosynthesis (29, 62). MptA (Rv2174) and MptB (Rv1459c) mannosyltransferases synthesize the characteristic $\alpha(1\rightarrow6)$ mannan core of LM and LAM. Specifically, in vitro assays in combination with deletion mutations in *C. glutamicum* characterized MptA as responsible for the synthesis of the distal end of the mannan core, whereas MptB was suggested to be involved in the proximal end of the mannan core (55, 90, 91). Although deletion of MptB in *C. glutamicum* resulted in a lack of LM and LAM and diminished $\alpha(1\rightarrow6)$ Man*p*T activity, complementation with MptB (Rv1459c) from *M. tuberculosis* or *M. smegmatis* failed to restore the wild-type phenotype of *C. glutamicum* (91). One could speculate that a slight variance in substrate specificity of the MptB orthologs may be responsible for this discrepancy. In addition, deletion of MptB in *M. smegmatis* had no effect on LM and LAM assembly, suggesting possible gene redundancy (91). Therefore, either an uncharacterized Man*p*T substitutes for MptB deletion or MptA is responsible for the biosynthesis of both proximal and distal ends of the mannan core. MptC (Rv2181) introduces the single $\alpha(1\rightarrow2)$ Man*p* residues of the mannan backbone, resulting in a mature LM (93). LAM is generated by the addition of 55–70 Ara*f* residues to LM, resulting in an arabinan domain similar to that of AG. It is speculated that a yet-unidentified Ara*f*T primes the mannan core with a few Ara*f* residues in similar fashion as AftA, which primes the galactan chain in AG (50, 92). EmbC (Rv3793) is exclusively responsible for the extension of primed LM with the addition of 12–16 Ara*f* residues in a linear $\alpha(1\rightarrow5)$ fashion (2, 114). AftC, a branching enzyme involved in the biosynthesis of AG, was demonstrated to introduce $\alpha(1\rightarrow3)$ Ara*f* residues in LAM (17, 18). A second branching $\alpha(1\rightarrow3)$ Ara*f*T AftD has been proposed to play a role in LAM biosynthesis; however, its clear function in LAM biosynthesis remains to be determined (116). The nonreducing arabinan domain of LAM is completed by addition of $\beta(1\rightarrow2)$ Ara*f* residues, resulting in characteristic tetra-arabinoside and hexa-arabinoside motifs (111). Given that AftB possesses $\beta(1\rightarrow2)$ Ara*f*T activity in AG biosynthesis, it is highly likely that it plays a similar

role in LAM biosynthesis. Unlike the arabinan domain in AG, the arabinan domain of LAM is modified with mannose residues (27, 99). CapA (Rv1635c) has been identified as an $\alpha(1\rightarrow5)$ Man*p*T, which utilizes PPM as the sugar donor and attaches the first Man*p* residue to the nonreducing arabinan termini of LAM (33). The subsequent attachment of at least one $\alpha(1\rightarrow2)$ Man*p* residue is catalyzed by MptC (Rv2181), which is also responsible for the addition of single $\alpha(1\rightarrow2)$ Man*p* residues to the $\alpha(1\rightarrow6)$ mannose backbone resulting in ManLAM (56). The enzymes required for the attachment of MTX and succinyl residues to the LAM remain to be established.

MYCOLIC ACIDS

Structure and Biosynthesis of Mycolic Acids

These long-chain α-alkyl-β-hydroxy fatty acids (C_{70-90}) form an integral component in the mAGP complex and contribute to the fluidity and permeability of the cell wall (72). There are three distinct types: α-Mycolic acids are found in greatest abundance and exist only in a *cis*-cyclopropane configuration, whereas methoxy mycolic acids and keto mycolic acids contain cyclopropane rings in either a *cis*- or *trans*-configuration with an adjacent methyl branch (**Figure 5**) (87, 127, 128). The biosynthesis of mycolic acids can be illustrated as five distinct stages. Primarily, the short-chain fatty acids (C_{16-24}) are synthesized in FAS-I (118). These saturated fatty acids can form the α-alkyl moiety (C_{24}) or can be extended by FAS-II to form the mero-chain (C_{56}). FAS-II is initiated by a condensation reaction catalyzed by FabH (β-ketoacyl-ACP synthase III) utilizing malonyl-AcpM and palmitoyl-CoA, yielding a β-ketoacyl-AcpM intermediate. This is then reduced by MabA (β-ketoacyl-ACP reductase) to produce β-hydroxyacyl-AcpM, which is then dehydrated by HadAB/BC (β-hydroxyacyl-ACP dehydratase) to produce a *trans*-2-enoyl-AcpM intermediate. This is then reduced by InhA (enoyl-ACP reductase) to produce an acyl-AcpM elongated by two carbons. The FAS-II cycle continues with further condensation, catalyzed by KasA/B (β-ketoacyl-ACP synthase), returning to the above via MabA, HadAB/BC, and InhA. This cycling continues until the acyl chain reaches C_{42-62}, forming the saturated long-chain meromycolate.

Various modifications are made to the saturated C_{42-62} chain following its synthesis via FAS-II. These modifications provide functional groups to the meromycolate scaffold by various methods, including *cis*-/*trans*-cyclopropanation, keto and methoxy groups via CmaA1-2-, MmaA1-4-, and PcaA-type enzymes (35, 41, 42). The modifications to the meromycolate chain have been suggested to vary the fluidity and permeability of the cell wall as well as provide protection from the host immune system during infection (131). The meromycolic acid chain (C_{42-62}) is activated for the Claisen-type condensation by the generation of a meromycolyl-AMP by FadD32 (fatty acyl-AMP ligase) (38). This meromycolyl-AMP substrate is linked to the α-alkyl short chain (C_{22-24}) in a reaction catalyzed by Pks13 to produce α-alkyl-β-keto-mycolic acid, which is subsequently reduced (120). The greatest abundance of mycolic acids within the cell envelope and cell wall is found as bound esters in mAGP and as trehalose mycolates. This process is catalyzed by a process of transport via MmpL3 and the antigen 85 complex (48).

FUTURE PERSPECTIVES

The biosynthetic pathways of the individual components of the cell wall of *M. tuberculosis* are now largely understood; however, a number of key enzymes in AG biosynthesis remain to be elucidated, as discussed above. Moreover, mechanisms that regulate and coordinate mAGP assembly to spatially arrange this complex within the cell envelope are still to be determined. Determining the translocation mechanism of various lipid-linked sugar substrates, such as

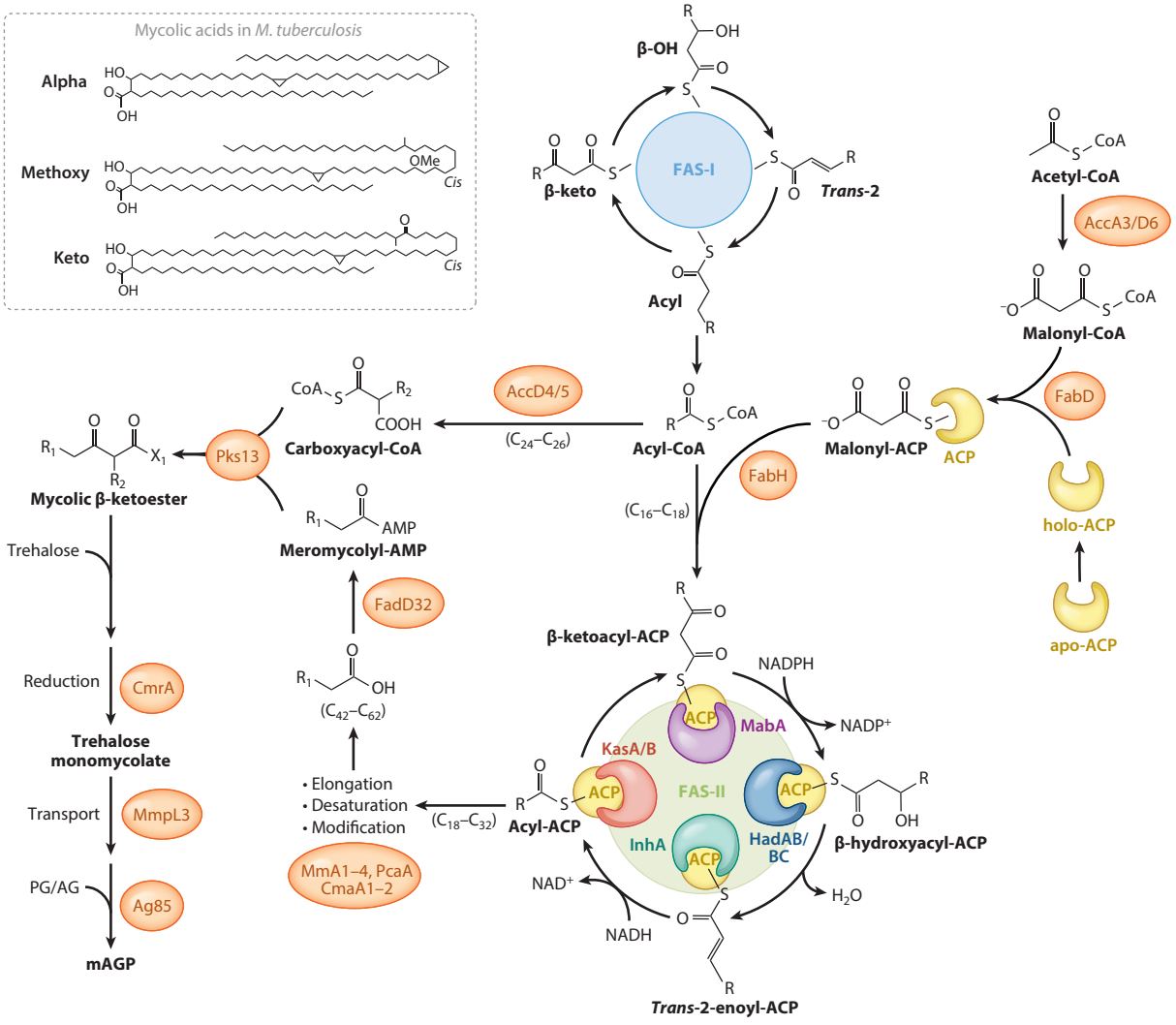

Figure 5

A schematic representation of mycolic acid biosynthesis in *Mycobacterium tuberculosis*.

intermediates involved in AG and LM/LAM biosynthesis, and sugar donors, DPA and PPM, may identify ideal drug targets. Although it is not clear how and when these intermediates are translocated to the periplasm, one could speculate that anchoring these molecules to the membrane positions them close to sugar transporters and glycosyltransferases, thus promoting export across the plasma membrane via multiprotein complexes. Recent studies employing a bacterial two-hybrid analysis demonstrated that enzymes involved in the biosynthesis of AG and precursor formation form complicated multiprotein complexes in *C. glutamicum* (49). Similar studies also reported that mycolic acid biosynthesis in *M. tuberculosis* is based on large multiprotein complexes, which are essential for mycobacterial viability, thus providing attractive drug targets (125, 126). Finally, structural and functional analysis of membrane proteins involved in cell wall

assembly lags behind that of soluble proteins involved in mAGP assembly. This is due to the inherent challenge of extracting pure membrane proteins from their native membrane in an active state. New approaches are attempting to address this limitation and will be key to the analysis and structural elucidation of mycobacterial membrane glycosyltransferases.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

G.S.B. acknowledges support in the form of a Personal Research Chair from Mr. James Bardrick. We also thank Dr. O.Y.-C. Lee, Dr. H.H.T. Wu, and Dr. L.J. Alderwick for their help in producing the structures of various lipids.

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